ANTI-CARCINOGENIC ACTIVITIES OF PROTEIN FROM PIPER LONGUM LINN IN VITRO STUDIES

Mr. Lucas K* | Dr. Aamarpali Roy**

**Ph.D. Scholar, Himalayan University, Itanagar, Arunachal Pradesh, India.*

***Research Supervisor, Himalayan University, Itanagar, Arunachal Pradesh, India.*

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ABSTRACT:

A major alkaloid, piperine is present in P.longum which showed significant anti-metastasis activity. Chemopreventive effects are also shown by piperine when it is administrated orally on the animals suffering from lung cancer. The fruits were air dried pulverized and used for analysis. Preliminary screening of secondary metabolites. Natural plants like long pepper are the main sources of bioactive molecules and have played a major role in discovery of lead compounds .The curative properties of medicinal plants of long pepper are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The bio enhancing effect of pepper species in traditional as well as modern medicine may be due to two major mechanisms; first, because of a nonspecific mechanism in which the rapid absorption of the drug may be observed by the decreased secretion of HCl. Despite the outstanding potential of piperine reported in pre-clinical studies, no clinical trials are ongoing in cancer patients. The bio enhancing properties of piperine are being explored in a clinical trial in association with curcumin to assess whether the combination may reduce inflammation and discomfort from a ureteric stent in cancer patients

Key Words: *P. longum, anticancer potential, cancer cell.*

ABOUT AUTHORS:

Author Mr. Lucas K is Research Scholar in Himalayan University, Itanagar, Arunachal Pradesh, India. He has attended various Seminars and conferences.

Author Dr. Aamarpali Roy is Research Supervisor in Himalayan University, Itanagar, Arunachal Pradesh, India.

INTRODUCTION:

Significant anti-tubercular activity is reported to exhibited by Piper longum. A major alkaloid, piperine is present in P.longum which showed significant anti-metastasis activity. Chemopreventive effects are also shown by piperine when it is administrated orally on the animals suffering from lung cancer. Cytotoxic activity is showed towards tumor cell lines by alkaloidal amides such as piplartine and piperine . The piperine and piplartine constituent of the plant was studied for the antitumor activity in the mice model. A significant reduction of tumor weight was observed in piplartine and piperine-treated animals. In another study, the alcoholic extract of P. longum and piperine at the dosage of 10 mg and 1.14 mg restricts the solid tumor development in mice induced with Dalton's lymphoma ascites cells and increases the life span of the mice model. The piperine component showed cytotoxicity in Dalton's lymphoma ascites and Ehrlich ascites carcinoma cells at the dosage of 250 mg/ml. Another study showed that the piperine constituent inhibits the lung metastasis induced by B16F-10 melanoma cells when studied in C57BL/6 mice, thus showed chemopreventive effects when administered orally. The oral administration of the ethanolic extract showed a protective effect on the cell surface and maintain the structural integrity of the cell membrane during DMBA induced hamster buccal pouch carcinogenesis. The previous studies demonstrated that the piperine constituent exhibits anti-apoptotic, anti-oxidative and restorative ability against cell proliferative mutagenic response.

MATERIALS AND METHODS

The schematic outline of methods for protein extraction from leaf. Each method was repeated four times to check the reproducibility. Method I is the modified TCA/Acetone method with some modification. Method II is the Dense SDS/Phenol method, Method III is the PVP/TCA acetone method with some modification. Method The present study was concentrated on the preliminary screening, qualitative screening of metabolites and anti-cancerous activity from the fruits of Piper longum Linn. The fruits were air dried pulverized and used for analysis. Preliminary screening of secondary metabolites: The shade dried plant material was powdered using mixer grinder and subjected to Soxhlet extraction with petroleum ether, chloroform, 95% ethanol and distilled water for 18 h in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening.

RESULTS

Aqueous Extract of Long Pepper (AQX) effectively and Selectively Reduces the Viability of & Induces Apoptosis in Cancer cells in a Dose & Time Dependent Manner

The first step in understanding the effect of long pepper extract in this study was to assess the effect of AQX on the viability of cancer cells. Following treatment with increasing concentration of AQX at increasing time points, cells were incubated with a water-soluble tetrazolium salt, which gets metabolized to a red formazan product by viable cells with active metabolism. This product can then be quantified by absorbance spectrometry. We observed the efficacy of crude AQX in reducing the viability of cancer cells, including colon (HCT116), pancreatic (BxPC-3), ovarian cancer (OVCAR-3) and melanoma cells. This effect was dose and time dependent. To further evaluate the anticancer activity of AQX, we wanted to assess its role in cell death and its selectivity to cancer cells. Our results demonstrate that AQX is able to selectively induce cell death in cancer cells (colon, pancreatic and leukemia) in a dose and time dependent manner, as characterized by the increase in propidium iodide positive cells in cancer cells treated with AQX. Furthermore, this effect was selective, as normal colon epithelial cells remained unaffected by this treatment, at the same concentrations and time-points. These results were quantified using image-based cytometry to determine the percentage of cells undergoing apoptosis and total cell death. We observed a 30–40% increase in annexin V positive cells, following PLX treatment and an 80–100% PI positive increase in the same cell samples, confirming the induction of apoptosis, following by necrosis in cultured cancer cells. DNA fragmentation is a key biochemical feature of apoptosis. To further confirm this induction of apoptosis, TUNEL labeling to detect DNA fragmentation was employed. Quantification results from image-based cytometry show the efficacy of PLX in inducing apoptosis, following DNA fragmentation in HT-29 colon cancer cells in a time dependent manner. VP16, a known chemotherapeutic agent with DNA damaging capabilities, was used as a positive control. Additionally, apoptosis induction in various cancer cells, melanoma (G-361), ovarian and colon cancer (HT-29) cells, was confirmed by Annexin-V binding assay. This induction of apoptosis was confirmed to be selective to cancer cells, as normal colon cells (NCM460) remained unaffected by PLX treatment. This was indicated by nuclear condensation, cell morphology and externalization of phosphatidyl serine to the outer leaflet of the cell membrane, as indicated by Hoechst staining, phase contrast images and binding of annexin V dye respectively. The selectivity of PLX to cancer cells was further confirmed by the WST-1 cell viability assay that showed that PLX was highly effective at such low doses, a therapeutic window was easily observed. Treatment of HT-29 with 0.20 mg/ml effectively reduced the viability by approximately 90%, while NCM460

DISCUSSION

Natural plants like long pepper are the main sources of bioactive molecules and have played a major role in discovery of lead compounds .The curative properties of medicinal plants of long pepper are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. A vast array of secondary metabolites was found to be present in the plant under study. A wide variety of essential oils were found to be present and best observed at 366nm. Number of coumarins and phenol carboxylic acids was found to be present in the plants. Compounds with Rf =0.17, Rf =0.25, Rf =0.29, and Rf =0.30 was observed to be best separated at 550nm, post derivatization. Anthraglycosides: Anthraglycosides was found to be best separated with optimum sample application of 10µl at 366nm. There is no need for derivatization since compounds are seen best separated before derivatization. Alkaloids were found to be best observed at 254nm with optimum sample application of 10µl. Spectra was observed at different Rf (0.64, 0.71, 0.76, 0.83). Sterols were found to be best observed at 366nm with optimum sample application of 10µl. The best compounds seen to be separated were at Rf = 0.18, Rf =0.26, Rf =0.31 and Rf = 0.65. Long pepper is widely used in the traditional (Ayurveda, Siddha, Unani, and Tibetan) system of medicine; and piperine, an active alkaloid present in pepper species is used as a bioenhancer. The bio enhancing effect of pepper species in traditional as well as modern medicine may be due to two major mechanisms; first, because of a nonspecific mechanism in which the rapid absorption of the drug may be observed by the decreased secretion of HCl. It also enhances the effect of gamma-glutamyl transpeptidase and by increasing the blood supply to the GI tract. Secondly, by inhibiting the enzymes involved in biotransformation drugs and decreasing the rate of elimination. It has been reported in vitro and in vivo interaction of piperine with the drug metabolizing enzymes present in hepatic tissues. The study revealed that, piperine was found as an inhibitor of aryl hydrocarbon hydroxylation (AHH), ethyl morphine- N- demethylation, and 7- ethoxycoumarin-Odeethylation in rat post mitochondrial supernatant in a dose-dependent manner

CONCLUSIONS

Despite the outstanding potential of piperine reported in pre-clinical studies, no clinical trials are ongoing in cancer patients. The bio enhancing properties of piperine are being explored in a clinical trial in association with curcumin to assess whether the combination may reduce inflammation and discomfort from a ureteric stent in cancer patients (ClinicalTrials.gov Identifier: NCT02598726). The synergistic or additional effects arising from the combination of chemotherapeutic agents and phytochemicals, of which P.longum is rich, represent a cutting-edge topic for anticancer research. However, human studies and clinical trials deepening the bio enhancing properties and anticancer effects of P.longum components alone and in association with anticancer drugs are missing, although crucial for supporting the efficacy and safety in cancer patients.

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